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Understanding Mushroom Virus X disease

Key external stakeholders:

Irish Mushroom Industry, Compost Companies, Research community

Practical implications for stakeholders:

There is a poor understanding of how Mushroom Virus X (MVX) induces the brown, off-coloured, poor-quality mushrooms associated with this new complex disease. The project aimed to advance our understanding of the disease and to develop diagnostic tests to facilitate its detection.

- Mushroom crops can be infected with MVX at any stage of the crop cycle but symptoms, and consequent financial losses, generally only develop when infection occurs at the end of the compost incubation phase (Phase 3).
- In order to prevent MVX symptoms developing, composters and growers need to ensure that there is no MVX-contamination of the compost (or casing) when incubated (Phase 3) compost is being filled into mushroom growing rooms.
- MVX can be reliably detected in mushrooms using a molecular diagnostic test.

Main results:

- **Critical infection time identified.** A low rate of MVX- infected material (0.01%) that is incorporated into compost or casing at the end of the compost incubation period gave the most consistent symptom expression. Thus it is imperative that mushroom compost and casing does not come into contact with any infective material (from previously infected crops) at this time.
- **MVX moves through compost very rapidly.** Following a point-infection of MVX into mushroom compost it was found to move at least 4 m (length of compost studied) within a single cropping period. This contrasts with a <1 m spread for fungal diseases following a point-infection.
- **Agronomic factors do not influence MVX symptoms expression.** Crops grown under stressful environmental conditions were no more likely to develop brown mushroom symptoms than crops grown under non-stressful conditions. MVX does not appear to be "triggered" by environmental factors.

Opportunity / Benefit:

The improved understanding of the epidemiology of Mushroom Virus X informs growers, composters, advisors and researchers of possible routes of transmission on facilities and allows for preventative measures to be taken.

Collaborating Institutions:

AFBI, Northern Ireland; DIT, Dublin; mushroom composters, growers and Allied Trades

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Commercial Mushroom Producers P.O. (CMP), mushroom composters, mushroom growers and Allied Trades

1. Project background:

Mushroom virus X (MVX) is a relatively new disease affecting the production of the commercial white mushroom, *Agaricus bisporus*, causing a range of disruptive symptoms within crops. The most predominate symptom in Ireland is the occurrence of 'brown' and off-coloured mushrooms in white strain crops. These mushrooms are rejected upon picking, after a period of cold storage, or by the retailer, and result in financial losses to growers. Other symptoms include crop delay, bare patches and poor quality or malformed fruit bodies. The epidemiology of the disease is not well understood but symptoms are associated with the presence of double-stranded RNAs (dsRNAs) in mushrooms of crops with symptoms. DsRNAs are indicative of replicating viral nucleic acids. A number of dsRNAs have been identified in mushrooms with symptoms. A range of low molecular weight dsRNAs (0.6-2.2 kb) are associated with the 'brown' symptoms, while a larger 3.6kb band is commonly found in mushrooms from crops experiencing delay and bare patches.
Anecdotal evidence has pointed to poor hygiene and/or crop management as critical factors in disease expression. While some control of the disease has been gained by increased hygiene measures, outbreaks continue to occur regularly but they are transient and inconsistent in nature.

2. Questions addressed by the project:

- What are the optimum conditions for MVX symptom expression?
- Does the source of MVX-infective material, i.e. the dsRNA band pattern of the infective material affect the range and severity of symptom expression?
- Can environmental and agronomic factors cause or influence symptom expression?
- Can the effect of symptoms on mushroom quality be quantified pre- and post-harvest?
- Can a reliable and effective molecular diagnostic be developed to test for MVX?

3. The experimental studies:

MVX-infected *Agaricus bisporus* isolates, containing different dsRNA banding patterns, were used to prepare MVX-inoculum for the various cropping experiments conducted by Teagasc, Kinsealy and AFBI, Loughgall. Cropping experiments at Kinsealy looked at the effects of different rates and times of MVX-infection on symptom expression, as well as the influence of different MVX dsRNA band profiles. Point inoculation cropping experiments were also conducted to see how rapidly MVX could move through incubated compost. Studies at Loughgall examined the effect of agronomic factors, such as ruffling, and environmental factors such as humidity, evaporation and watering management, to provide "stressful" conditions that might trigger or influence the expression of MVX symptoms. Mushroom "colour" was measured quantitatively using a colorimeter to quantify the level of brown and off-coloured mushrooms observed in all crop experiments. Measurement of post harvest quality was done at DIT using a wide array of analytical tools, techniques and methodologies such as Colorimetry, RGB image acquisition, hyperspectral imaging, Fourier Transform Infra Red microscopy (FT-IR). External quality indexes (colour, density, weight loss) were analysed using wet lab and image analysis together with biochemical quality indexes (enzyme activity, browning pigments).
Finally, in order to confirm MVX presence in both mushrooms and compost, reliable diagnostic tests were developed using standard and newly-reported molecular biology techniques for nucleic acid extraction, purification, electrophoresis, and PCR.

4. Main results:

- **Critical infection time identified.** The critical infection time and conditions that lead to consistent appearance of MVX brown mushroom symptoms were identified. A low rate of infected material (0.01%) that is incorporated into compost or casing at the end of the compost incubation period gave the most consistent symptoms. Thus it is imperative that mushroom compost and casing does not come into contact with any infective material (from previously infected crops) at this time. Composters and growers should

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enhance hygiene measures to minimise any cross-contamination at this critical time.

- **MVX moves through compost very rapidly.** Following a point-infection of MVX into mushroom compost it was found to move at least 4 m (length of compost studied) within a single cropping period. This contrasts with a <1 m spread for fungal diseases following a point-infection.
- **Agronomic factors do not influence MVX symptoms expression.** Crops grown under stressful environmental conditions were no more likely to develop brown mushroom symptoms than crops grown under non-stressful conditions. MVX does not appear to be "triggered" by environmental factors.
- **Effective molecular diagnostic test to detect MVX in mushrooms.** A PCR based diagnostic test for mushrooms was effective and reliable, detecting MVX in infected mushrooms. MVX was also detected in mushrooms and crops that showed few/no symptoms. An industry-wide survey also detected MVX at a level higher than the occurrence of symptoms suggested. This would explain the persistence and transience of the symptoms within the Irish industry since the late 1990's. Although a test to detect MVX in compost was developed it was only not entirely reliable due to the humic acid content of compost.
- **FT-IR detection of MVX in post-harvest mushrooms shows promise**. Detection of MVX in post-harvest mushrooms using spectroscopic methods and its discrimination from other causes of browning proved difficult but the FT-IR micro-imaging method shows promising results. This technique may be useful to rapidly detect MVX within a few hours in mushroom samples and might develop into a complementary technique to the molecular diagnostic.

5. Opportunity/Benefit:

The research results from this project have provided both the Irish and International mushroom community with a substantial body of information on how MVX infects and affects a mushroom crop. The most critical infection time has been identified, highlighting the need for excellent hygiene measures to be in place to prevent crops becoming contaminated. The research also highlighted the fact that non-symptomatic MVX infections are common, which is likely to be the reason why the disease is so persistent and has been so hard to control. This increase in understanding has paved the way for future research to focus on how to monitor for, detect and eliminate MVX-infected material on mushroom farms and compost facilities. Industry stakeholders across Europe have acknowledged Teagasc's expertise in this area leading to a major International collaborative EU FP7 project being funded in 2012 (www.MushTV.eu).

6. Dissemination:

The outcomes of this research have been disseminated to mushroom composters and key staff through a series of Internal Seminars on individual premises during 2011. Mushroom growers and their staff were reached via Disease Control seminars in 2011 and 2012, which were organised by CMP, in conjunction with Teagasc, at venues in Cavan, Monaghan, Westmeath and Tipperary. In addition the key results were presented to a wider audience at the 2011 All Ireland Mushroom Conference in the Hillgrove Hotel in Monaghan (21 October 2011) via a presentation, posters and informal contact with growers during the event. (http://www.teagasc.ie/publications/2011/1056/index.asp). The Mushroom conference is a biennial event sponsored by Bord Bia in conjunction with Teagasc and key stakeholders. Teagasc advisory staff deal with grower queries on this topic and, when necessary, farm visits are made to provide one to one advice tailored for specific farms. A technical service can be provided to detect MVX in mushroom and compost samples.

Formal links with Industry: Work on MVX epidemiology and control continues with national and European industry and scientific partners via EU FP7 Project MushTV (6270) 2012- 2014 (www.MushTV.eu)

Main publications:

Grogan, H. (2011). Mushroom Virus X disease: Understanding the factors which trigger "brown mushroom" symptom expression as a means to improved diagnosis and control. Final Report RSF 07547. DAFF. http://www.agriculture.gov.ie/media/migration/research/rsfallfundedprojects/2007projects/RSF07547310712.pdf

Fleming-Archibald, C., Ruggiero, A., Kilpatrick, M. & Grogan, H.M. (2010). Mushroom Virus X Disease: A Whiter Shade of Pale Brown? T Research, 5: 14-15. http://www.teagasc.ie/publications/view_publication.aspx?publicationID=51

Fleming-Archibald, C., Ruggiero, A, & Grogan, H.M. (2009). Identifying factors which influence the expression of "brown mushroom" symptoms in crops infected with Mushroom Virus X. Agricultural Research Forum, 2009, p 124. http://www.agresearchforum.com/publicationsarf/2009/proceedings2009.pdf

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